β-SECRETASE ENZYME COMPOSITIONS AND METHODS

is a continuation of U.S. Application Number 09/501,708, now abandoned, and

This application claims the benefit of U.S. Provisional Application Numbers 60/119,571 filed 2/10/1999 and 60/139,172 filed 6/15/99, both of which are hereby incorporated herein by reference in their entireties.

MF 09/01/05

Field of the Invention

The invention relates to the discovery of various active forms of β-secretase, an enzyme that cleaves β-amyloid precursor protein (APP) at one of the two cleavage sites necessary to produce β-amyloid peptide (Aβ). The invention also relates to inhibitors of this enzyme, which are considered candidates for therapeutics in the treatment of amyloidogenic diseases such as Alzheimer's disease. Further aspects of the present invention include screening methods, assays, and kits for discovering such therapeutic inhibitors, as well as diagnostic methods for determining whether an individual carries a mutant form of the enzyme.

Background of the Invention

Alzheimer's disease is characterized by the presence of numerous amyloid plaques and neurofibrillatory tangles present in the brain, particularly in those regions of the brain involved in memory and cognition. β-amyloid peptide (Aβ) is a 39-43 amino acid peptide that is major component of amyloid plaques and is produced by cleavage of a large protein known as the amyloid precursor protein (APP) at a specific site(s) within the N-terminal region of the protein. Normal processing of APP involves cleavage of the protein at point 16-17 amino acids C-terminal to the N-terminus of the β-AP region, releasing a secreted ectodomain, α-sAPP, thus precluding production of β-AP. Cleavage by β-secretase enzyme of APP between Met ^{671} and Asp^{672} and subsequent processing at the C-terminal end of APP produces Aβ peptide, which is highly implicated in the etiology of Alzheimer's pathology (Seubert, *et al.*, *in* Pharmacological Treatment of Alzheimer's disease, Wiley-Liss, Inc., pp. 345-366, 1997; Zhao, J., *et al.* J. Biol. Chem. 271: 31407-31411, 1996).

It is not clear whether β-secretase enzyme levels and/or activity is inherently higher than normal in Alzheimer's patients; however, it is clear that its cleavage product, Aβ peptide, is abnormally concentrated in amyloid plaques present in their brains. Therefore, it would be desirable to isolate, purify and characterize the enzyme responsible for the